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Supplementary appendix

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Ancestral and Beta SARS-CoV-2 antibody seroprevalences in Malagasy blood donors during the spring 2021 COVID-19 epidemic

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Supplementary Material

Methods

Sample Collection
Plasma samples were collected at the Blood Transfusion Centre (RBTC) of Antananarivo, Madagascar as previously described[1].
Samples were collected twice a month starting in March 2020 at RBTC Antananarivo. For each sample, the blood donor's gender, age, occupation and date of collection were collected.
Plasma samples from Antananarivo were aliquoted at the Joseph Ravoahangy Andrianavalona Hospital (HJRA) in 1mL cryotubes, then stored at -20°C, and analyzed at the Infectious Diseases Immunology Unit of the Pasteur Institute of Madagascar.
Duplicates as well as samples from donors under the age of 18 were eliminated from the study. All Collected samples were analyzed.

Serology
Semi-quantitative indirect ELISA for detection of anti-SARS-CoV-2 Antibodies
The ID Screen® SARS-CoV-2-N IgG Indirect ELISA (ID.Vet, Grabels, France) of semi-quantitative type, demonstrates the antibodies (IgG) directed against the nucleocapsid (N) of the SARS -CoV-2 in human serum or plasma. Its use has been previously described[1].
In addition to the ID Screen kit, some samples were tested with the semi-quantitative WANTAI SARS-CoV-2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.) kit.
The WANTAI kit demonstrates the IgA, IgG, IgM antibodies directed against the Spike RBD antigen of SARS-CoV-2. These antibodies are more persistent and allow counting both recently infected patients and those infected almost a year ago. According to the manufacturer, the kit has a specificity of 100% and a sensitivity of 94.36%. We found a 95% Se at 6 months of diagnosis on an independent cohort (data not shown).

Variant serology
Luminex Xmap technology, a type of immunoassay that uses magnetic beads to simultaneously measure multiple analytes in a single experiment, was used to determine the presence of donor antibodies to ancestral or mutated variant proteins.
Briefly, the samples to be tested were diluted to 1/100 and 100uL were distributed in the specific well and then, incubated with MAGPLEX COOH-microsphere beads (MC10012-01, Luminex) coupled either to ancestral proteins (SARS-CoV-2 Spike protein-RBD-HisTag, Z03483, GenScript) or to proteins comprising mutations specific to B.1.351 (SARS-CoV-2 (2019-nCoV) Spike RBD (K417N, E484K, N501Y)-HisRecombinant Protein, 40592-

V08H85, SinoBiological, Beijing, P.R. China). After 30 min of incubation, magnetic microspheres were incubated 60s on magnet plate and washed with assay buffer (PBS 0.05%, BSA 0.1%, pH7.4). After a washing step, a phycoerythrin labelled protein G conjugate (H10104, Thermofisher, Massachusetts, U.S.) recognizing IgG is distributed into the wells. This binds to IgGs, forming an antigen-antibody-conjugate-PE complex.
After removal of the excess conjugate by washing, the microplate is read in a Magpix instrument (MAGPX12234702, Texas, USA). The beads are then identified, and the PE fluorescence quantified. The amount of specific antibodies present in the sample is proportional to the intensity of fluorescence emitted. It is then possible for each sample to know whether the IgG binds more or less to ancestral RBD or to RBD containing mutations specific to B.1.351 calculating a $RBD^{B.1.351}/RBD^{WT}$ MFI ratio, and therefore predict exposure to B.1.351.

Threshold definition
Sample collected in October 2020 were used for the calculation of the cutoff as variant B.1.351. was first identified in October 2020 after excluding an outlier (n=1) by Normality test (Shapiro–Wilk test). $RBD^{B.1.351}/RBD^{WT}$ MFI cut off values were determine using the "Mean + 3SD" formula (=0.58233785).

All recent seropositive samples (Anti-N IgG^{+}) from October to May 2021 were analyzed.

Statistical analysis
Analysis was using GraphPad Prism version 8.0 (GraphPad, La Jolla, CA). Statistical significance was calculated using *chi square* test, confidence intervals calculated using Wilson/Brown method.

Ethics
This study was authorized by the Ministry of Health of Madagascar and by the BioMedical Research Ethics Committee (CERBM) that informed the investigators it required neither the approval of the committee nor specific donor consent (CERBM: IOR0000851) (Authorization N°205-MSANP/SG/AGMED/CERBM). Blood donors are advised their blood may be tested for surveillance purposes.

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The contents are the responsibility of the authors and do not necessarily reflect the views of USAID and the United States Government.

Supplementary Table 1: Demographic characteristics of the study population included from October 2020 to May 2021

Collection month	Number of collected samples	Mean age	Gender ratio	Men %	Women %	Missing information
October	497	34 [18-61]	2,7	72.6%[361/497]	27.2%[135/497]	0.2%[1/497]
November	503	33 [18-64]	2,9	74%[372/503]	25.8%[130/503]	0.2%[1/503]
December	177	31 [18-59]	3,7	78.5%[139/177]	21.5%[38/177]	0%[/177]
January	389	32 [18-62]	2,5	71.7%[279/389]	28.3%[110/389]	0%[/389]
February	319	34 [18-62]	2,7	73%[233/319]	26.6%[85/319]	0.3%[1/319]
March	497	33 [18-61]	2,9	73.8%[367/497]	25.8%[128/497]	0.4%[2/497]
April	493	32 [18-61]	3,4	77.3%[381/493]	22.7%[112/493]	0%[/493]
May	500	33 [18-68]	2,5	71.2%[356/500]	28.4%[142/500]	0.4%[2/500]
TOTAL	**3375**	**33 [18-68]**	**2,8**	**73.7%[2488/3375]**	**26.1%[880/3375]**	**0.2%[7/3375]**

Supplementary Table 2: Monthly anti-N IgG and anti-RBD total Ig seroprevalences in the Antananarivo (Analamanga region) National blood transfusion centre blood donors and B.1.351 positivity rates in anti-N IgG positive samples (* samples only analysed for anti-RBD total Ig starting in January 2021)

Collection month	collected samples (N)	Anti-N IgG seroprevalence			Anti-RBD Igs seroprevalence			B.1.351 Positivity rate		
		N	(%)	(95% CI)	N	(%)	(95% CI)	N	(%)	(95% CI)
October	497	177	35.8	31.7 – 40.1	– *	– *	– *	2	1.1	0.2 – 4.0
November	503	153	31.2	27.3 – 35.4	– *	– *	– *	3	2.0	0.5 – 5.6
December	177	39	22.0	16.6 – 28.7	– *	– *	– *		0.0	0.0 – 0.0
January	389	66	17.0	13.6 – 21	174	44.7	39.9 – 49.7	3	4.5	1.2 – 12.5
February	319	54	17.2	13.5 – 21.8	161	50.5	45.0 – 55.9	3	5.6	1.5 – 15.1
March	497	59	15.9	12.9 – 19.4	248	49.9	45.5 – 54.3	12	15.4	9.0 – 25.0
April	493	145	29.2	25.4 – 33.4	288	58.4	54.0 – 62.7	79	54.5	46.4 – 62.4
May	500	236	47.6	43.3 – 52.0	384	64.8	56.1 – 72.6	149	63.1	56.8 – 69.0

N, number of positive samples

Contributors:

Conceptualisation and methodology: MS, SLR, LTR, MDBN. Investigation: SLR, MS, PD. Formal analysis: MS and SLR. (LTR, MDBN and MS have verified the underlying data). Resources and funding acquisition: MS, VR, ZAR, AS. Supervision: MS, ZAR, AS. Writing – Original draft preparation: MS, SLR, MDBN, LTR. Writing – Review and editing: all authors.

Declaration of Interests:

All authors declare no competing interests.

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Data sharing: Data will be made available with publication (All data and deidentified participant age and gender) upon request to the corresponding author.